PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 February 2001 (14.02.01)	
International application No. PCT/EP00/05066	**Applicant's or agent's file reference** SCB557PCT
International filing date (day/month/year) 02 June 2000 (02.06.00)	**Priority date** (day/month/year) 03 June 1999 (03.06.99)
Applicant BARTORELLI, Alberto	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 December 2000 (21.12.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
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1231

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB557PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/05066	02/06/2000	03/06/1999
International Patent Classification (IPC) or national classification and IPC A61K38/44		
Applicant PHARMAPRODUCTS UK LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/12/2000	16.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Langer, A Telephone No. +49 89 2399 7809

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/05066

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-4 as originally filed

Claims, No.:

1-3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-3
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-3
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-3
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: R.J. COOK ET AL.: 'ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR RAT LIVER 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 8, 15 March 1991 (1991-03-15), pages 4965-4973, XP002157451 BALTIMORE, MD, US cited in the application

2. The present application refers to the use of 10-formyltetrahydrofolate dehydrogenase as therapeutical agent, more particularly as anti-tumor agent.

3. Document D1 discloses the cDNA sequence of rat 10-formyltetrahydrofolate dehydrogenase, also indicating that it could serve to investigate physiological role of this enzyme (last paragraph).

4. **Novelty (Art. 33 (2) PCT) and Inventive Step (Art. 33 (3) PCT)**

   The prior art does not contain any indication for the use of 10-formyltetrahydrofolate dehydrogenase as a therapeutical agent. The subject-matter of **claims 1-3** therefore fulfills the requirements of Art. 33 PCT in terms of novelty and inventive step.

5. **Industrial Applicability (Art. 33 (4) PCT)**

   For the assessment of the present **claims 1-3** on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such

a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 00/73330	07/12/2000	01/06/2000	01/06/1999

Although document WO 00(733330 is not prior art according to R. 64.1(a) PCT, it discloses the subject-matter of **claim 1** (claims 34-38). This document may therefore in some contracting states be relevant for the evaluation of the present application.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74711 A2

(51) International Patent Classification[7]: A61K 38/44, A61P 35/00

(21) International Application Number: PCT/EP00/05066

(22) International Filing Date: 2 June 2000 (02.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI99A001243 3 June 1999 (03.06.1999) IT
MI99A002197 20 October 1999 (20.10.1999) IT

(71) Applicant *(for all designated States except US)*: **PHARMAPRODUCTS UK LIMITED** [GB/GB]; Castle Chambers, 7th Floor, 43 Castle Street, Liverpool, Merseyside L2 9TL (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **BARTORELLI, Alberto** [IT/CH]; Chalet Christina - Bois Doré, Chemin des Biolirs, CH-3963 Crans-sur-Sierre (CH).

(74) Agents: MINOJA, Fabrizio et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 08, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/74711 A2

(54) Title: 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

(57) Abstract: The use of 10-formyltetrahydrofolate dehydrogenase as therapeutical agent, in particular as cytotoxic and antitumour agent, and the process for the preparation thereof.

10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

The present invention relates to the use of 10-formyltetrahydrofolate dehydrogenase as therapeutical agent, in particular as cytotoxic and antitumour agent.

10-Formyltetrahydrofolate dehydrogenase is an enzyme present in the liver and in the nervous system of mammals. No therapeutical use for such enzyme has been disclosed up to now.

cDNA from rat 10-formyltetrahydrofolate dehydrogenase has been disclosed in J. Biol. Chem. 266(8), 4965-4973, 1991, while cDNA of the same human enzyme has been disclosed more recently (Biochem. Mol. Biol. Int., 47(3), 407-415, 1999).

Furthermore, methods for the preparation of the recombinant enzyme are known from Protein Expression Purif. 6, 457-64, 1995 and Biochem. J. 306(3), 651-5, 1995.

It has now been found that mammal 10-formyltetrahydrofolate dehydrogenase is capable of inducing a marked cytotoxic response against tumour cells, when administered to tumour-bearing patients or animals.

This cytotoxicity seems to be mediated by cytotoxic antibodies to human tumour cells, particularly carcinomas and adenocarcinomas.

Cytotoxicity can be quantified in vitro on Jurkat and Kato III cells using conventional methods, based for example on the use of commercial kits such as the CDC-UK kit (Pharmaproduct). In particular, the appearance of cytotoxicity in rabbits serum was observed already after a first treatment with the enzyme (1 mg/animal in saline solution) on Jurkat and Kato III cells.

Therefore, the invention also relates to pharmaceutical compositions containing as active ingredient

an effective dose of 10-formyltetrahydrofolate dehydrogenase.

The compositions of the invention will be administered to tumour patients using the conventional administration routes for proteins and polypeptides, for example the subcutaneous or intramuscular routes. The treatment may be repeated, a treatment comprising one-two week separated administrations of doses ranging from 0.1 to 20 mg of enzyme being preferred.

Furthermore, it has surprisingly found that it is possible to induce high cytotoxicity by administering the enzyme even at very low dosages, such as 1.10^{-4} - 1.10^{-10} g, through the sublingual route, in the form of granules or drops of 1% water-alcoholic solutions or suspensions in ethanol, with concentrations of active ingredient ranging from 10^{-6} to 10^{-10} M.

10-Formyltetrahydrofolate dehydrogenase can be prepared by conventional recombinant DNA methods or it can be extracted from the liver of animals, for example from liver of bovine, ovine or swine. Goat liver proved to be a particularly abundant source of this enzyme.

The extraction process comprises the treatment of livers with solutions buffered at pH 7.4 (PBS) followed by precipitation with 15% polyethylene glycol 6000, chromatography on TSK-DEAE or DEAE-Sephacell at pH 8, elution with 0.3 M NaCl and purification on TSK SW3000.

The following example illustrates the invention in greater detail.

EXAMPLE

Extraction

50 g of goat liver are homogenized, suspended in 400 ml of PBS 0.01 M pH 7.2, stirred for 30 minutes at 4°C and centrifuged on JA14 at 14,000 RPM for 30 minutes. After that, the product is filtered with suction; then through

1.2 μm filter, finally through 0.45 μm filter.

Volume: 340 ml conc. 10.9 mg/ml.

Fractional precipitation with PEG 6000.

336 ml of the above sample are treated with 5% powder PEG 6000 (16.8 g). The whole is stirred for 1 hour at 4°C, then centrifuged on J 6 at 4,000 g for 30'.

The pellet is taken up into 61 ml of 0.03M Tris/HCl pH 8, whereas the supernatant (340 ml) is reprecipitated with 5% PEG 6000 (17 g), then 10% PEG 6000 stirring for 1 hour at 4°C.

After centrifuging on J6 at 4,000 g for 30', the pellet is taken up with 62 ml of 0.03M Tris/HCl pH 8.

The supernatant (345 ml) is treated with 5% PEG 6000 (17.25 g), then again with 5% PEG 6000, stirring for 1 hour at 4°C, then centrifuged on J6 at 4,000 g for 30'.

The supernatant is discarded, and the pellet is taken up into 200 ml of 0.03M Tris/HCl pH 8.

5% PEG pellet volume: 61 ml, conc. 9.34 mg/ml.

10% PEG pellet volume: 62 ml, conc. 13 mg/ml.

15% PEG pellet volume: 200 ml, conc. 3.38 mg/ml.

DEAE - Sephacell

About 150 ml of DEAE-S resin are equilibrated in 0.03 M Tris/HCl buffer pH 8. The resin is incubated with the 15% PEG sample for 30 minutes at room temperature + 200 ml of washing.

Leg 1: 200 ml 0.5M Tris/HCl pH 8 for 30 minutes at r.t. + 200 ml of washing.

Leg 2: 200 ml 0.03M Tris/HCl pH 8 + 0.3M NaCl for 30 minutes at r.t. + 200 ml of washing.

Leg 3: 200 ml 0.03M Tris/HCl pH 8 + 1M NaCl for 30 minutes at r.t. + 200 ml of washing.

The following samples are thereby obtained:

S.B. volume: about 400 ml conc: 294 ✓/ml.

LEG 1 volume: about 400 ml conc: 1.14 mg/ml.

LEG 2 volume: about 400 ml conc: on PM 30.

LEG 3 volume: about 400 ml conc: 137 γ/ml.

LEG 2 is conc. on PM 30 to an about 20 ml final volume, concentration of about 3.6 mg/ml.

SW3000 prep.

LEG 2 from DEAE-S obtained above is purified in prep. SW3000 prep. (10 runs, 2 ml each).

Four fractions are eluted, the second being concentrated on PM 30 and dyalised against H_2O to a final volume of about 2 ml, concentration of about 1.5 mg/ml.

CLAIMS

1. 10-Formyltetrahydrofolate dehydrogenase as therapeutical agent.

2. 10-Formyltetrahydrofolate dehydrogenase as antitumour agent.

3. The use of 10-formyltetrahydrofolate dehydrogenase for the preparation of cytotoxic and antitumour medicaments.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

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(51) International Patent Classification[7]: A61K 38/44, A61P 35/00 // C12N 9/02

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI99A001243 3 June 1999 (03.06.1999) IT
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(71) Applicant *(for all designated States except US)*: **PHARMAPRODUCTS UK LIMITED** [GB/GB]; Castle Chambers, 7th Floor, 43 Castle Street, Liverpool, Merseyside L2 9TL (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **BARTORELLI, Alberto** [IT/CH]; Chalet Christina - Bois Doré, Chemin des Biolirs, CH-3963 Crans-sur-Sierre (CH).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 08, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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26 July 2001

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WO 00/74711 A3

(54) Title: 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

(57) Abstract: The use of 10-formyltetrahydrofolate dehydrogenase as therapeutical agent, in particular as cytotoxic and antitumour agent, and the process for the preparation thereof.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 00/05066

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/44 A61P35/00 //C12N9/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, CHEM ABS Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R.J. COOK ET AL.: "ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR RAT LIVER 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 8, 15 March 1991 (1991-03-15), pages 4965-4973, XP002157451 BALTIMORE, MD, US cited in the application figure 3 ---	1-3
E	WO 00 73330 A (PROTEOME SIENCES PLC) 7 December 2000 (2000-12-07) page 91, marker "LOM17" claims 34-38 ----	1,2

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2001	22/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/EP 00/05066

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0073330 A	07-12-2000	GB 2350676 A	06-12-2000